REMARKS

With entry of this amendment, Claims 5 - 10, 12, 14, 15, 27 - 40 and 52 - 59 are pending. Claims 6 - 8, 14 and 27 have been amended and claims 58 - 66 are new. Support for the new and amended claims is at least found at page 12, lines 5 and 6. Claims 6-8, 14, and 27 have been amended to recite that the truncated pullulanse comprises a conserved Y region. Specifically support is found at page 8, lines 7-25, and page 12, lines 5 – 13 Claim 14 was further amended to recite the nucleic acid having at least 90% identity to the polynucleotide sequence as shown in SEQ ID NO:1. Specifically support is found at page 8, lines 7-14. New claims 58 and 59 depend on allowed Claims 9 and 10, respectively, and recite that the pullulanase is obtained from a *Bacillus deramificans* having the designation T89.117D in the LMG collection. Specifically support is found at page 9, lines 25-30. Additionally, claims 60-64 recite that the truncated pullulanase comprises a conserved VWAP region, which is defined at page Page 12, lines 5-14. Additionally, claims 65-66 depend on allowed claims 30 and 31, respectively, and recite that the compositions of claims 31 and 32 further comprise an enzyme selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds. Specifically support is found at page 5, lines 15-20.

Claims 9 10, 12, 31 and 32, have been allowed and Claims 5 – 8, 14, 15, 27-30, 33-40 and 52 – 57 were rejected in the Office Action dated January 27, 2003. Claim 55 was rejected under 35 USC § 112, second paragraph as failing to distinctly claim the invention. Claims 5-8, 14, 15, 27 – 30, 33 - 40 and 52-57 remain rejected under 35 USC § 103 as unpatentable over Deweer et al. (US 6,074,854), and Albertson et al.(Biochim.Biophys. Acta, Vol. 1354:35-39 [1997]), or McPherson et al. (Biochemical Soc. Trans., 1988, Vol. 16(5):723-724). Applicants assert neither the previous claims nor the present claims are rendered unpatentable by the references when taken alone or in any combination.

REJECTION 35 USC § 112

Claim 55 was rejected under 35 USC § 112, second paragraph as being indefinite as the phrase "wherein the deletion is obtained from a pullulanase" was unclear to the Examiner. Claim 55 has been amended as suggested by the Examiner. Applicants respectfully submit that the rejection has been overcome and the Section 112 rejection of Claim 55 should be reconsidered.

REJECTIONS UNDER 35 U.S.C. §103(a).

Claims 1, 3, 5 - 8, 11, 13 - 30 and 33 - 40 stand rejected under 35 U.S.C §103(a) as being unpatentable over Deweer et al. (US Patent No. 6,074,854) and McPherson et al. (Biochem. Soc. Trans., 1988, Vol 16(5):723-724) or Albertson et al. (Biochim Biophys. Acta. 1997, Vol. 1354(1): 35-39). Applicants respectfully traverse this rejection.

Applicants assert Deweers et al. is concerned with a full-length pullulanase derived from *B. deramificans* T 89.117D. The mature full length pullulanase has 928 amino acids as shown in SEQ ID NO: 11. At column 5, lines 36 - 65 the reference discloses "[T]he invention also relates to the isolation and provision of a DNA molecule comprising the nucleotide sequence (SEQ ID NO: 10) which codes for the pullulanase of *B. deramificans* T 89.117D (LMG P-13056) or a modified sequence derived therefrom ....." Further it is stated, "[T]he invention also relates to a modified pullulanase, that is to say an enzyme in which the amino acid sequence differs from that of the wild enzyme by at least one amino acid."

Applicants contend the disclosure in Deweers et al. does not teach a truncated pullulanase. While arguendo, Deweers et al. disclose that the wild-type pullulanase could be modified, there is no teaching whatsoever, of a truncated pullulanase as claimed in Applicants' application, wherein said truncated pullulanase comprised a conserved Y region alone or in combination with a conserved VWAP region. What Deweers et al. teach is that the pullulanase is synthesized in the form of a precursor protein wherein the signal sequence includes 29 amino acids (Column 5 lines 59-61 and Column 14, lines 1-23). Generally signal sequences are eliminated during the exportation of an enzyme to the outside of the cell and is not part of the mature protein. The removal of the signal peptide to obtain a mature enzyme does not comprise a truncated enzyme as claimed by the Applicants. There is no hint or suggestion of a conserved Y or conserved VWAP region as recited by the amended claims.

The Deweer et al. reference has been combined with McPherson et al. or Albertson et al. Applicants respectfully submit that neither McPherson et al. or Albertson et al. teach a truncated *Bacillus* pullulanase which comprises the conserved Y region nor which comprises a conserved VWAP region.

McPherson et al. teach the modification of deleting about 170 amino acid residues from the amino terminal end of *K. pneumoniae* pullulanase wherein the modification leads to higher activity as compared to the native enzyme (0.81 unit/mg vs 0.58 unit/mg). There is no hint or suggestion of the truncated pullulanase comprising a conserved region Y nor a conserved region VWAP. Rather than teach those skilled in the art the conserved regions, McPherson states, "we are examing further protease-generated variants and are constructing DNA deletion

derivatives to allow more accurate definition of an active 'core' pullulanase molelcue." Page 723, last line. Applicants respectfully submit that McPherson does not teach those skilled in the art of the truncated pullulanase comprising a conserved Y region. Thus assuming arguendo that there is a hint or suggestion of combining Deweer et al. with McPherson, it does not obviate the deficiency of Deweer, et al., that is that there is no hint or suggestion of the truncated pullulanase comprising a conserved Y or conserved VWAP region.

Albertson et al. discloses a recombinant plasmid pNZ1452, which Includes a 381bp deletion of the 5'region of a *Caldicellulosiruptor saccharolyticus* pullulanase. The plasmid when cloned into *E. coli* produced a pullulanase missing the first 95 amino acid residues but still able to hydrolyze pullulan. Thus assuming arguendo that there is a hint or suggestion of combining Deweer et al. with Albertson et al., it does not obviate the deficiency of Deweer, et al., no hint or suggestion of the truncated pullulanase comprising a conserved Y or conserved VWAP region.

The Examiner states,

> "It would have been obvious to one skilled in the art at the time the invention was made to combine the teachings of Deweer et al. with that of McPherson et al. or Albertson et al. to make a modified pullulanase in which N-terminal amino acids have been deleted. This is because Deweer et al teach a pullulanase isolated from a *Bacillus*, *B. deramificans*, which is very large enzyme with more than 900 amino acids. McPherson et al. teach a method of increasing the efficiency of large size pullulanase by determining and deleting non-essential amino acids in the N-terminal region and Albertson et al. and McPherson et al. teach that deletion of up to at least 100 - 300 amino acids does not affect the activity of the enzyme negatively but on the other hand increase the efficiency of the enzymes by nearly 30%."

However, Applicants contend the combination of references does not contain a sufficient teaching of how to obtain a truncated pullulanase from a *Bacillus* species wherein the truncated enzyme comprises a conserved Y region and retains the capacity to hydrolyze alpha-1,6-glucosidic bonds.

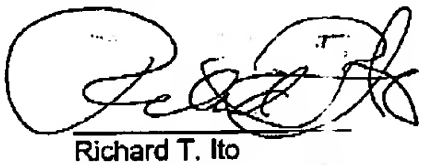
As presently amended, Claims 6-8, 14 and 27 recite a truncated pullulanase comprised of a conserved Y region. New Claims 60-64 recite a truncated Bacillus pulluanase further comprising a conserved VWAP region. Both of these terms are defined on page 12 of the specification. On page 12, the inventors believed that these two newly identified conserved regions indicated the limits of the amino acid truncations in the N-terminal of pullulanases. This belief was based on the lack of further conserved regions of identity among the known

pullulanases beyond the Y region as one proceeds to the N-terminus. As taught at page 8 of Applicants' specification, the deletion in the amino terminal amino acids of a *Bacillus* pullulanase can be of varying length, but is at least three amino acids in length and the deletion can go no further than the beginning of the first conserved domain which in *B. deramificans* is the tyrosine at amino acid residue 310 as shown in Figure 1. Also as disclosed by the Applicants at page 12 of the specification, Albertson et al. reveal the regions called DPY, A, B, C, D, E, and YNWGY as conserved regions among a group of gram-positive and gram-negative pullulanases. Two regions, DPY and YNWGY were identified as being characteristic of true pullulanases. In addition to the conserved regions highlighted by Albertson et al., Applicants significantly disclose two other conserved regions closer to the N-terminus of pullulanase. These regions are referred to as Y and VWAP and reference is made Figures 2A – 2D of the specification. These regions are not taught or suggested by Albertson et al., as being conserved regions. Applicants further disclose that the limits of amino acid truncations in the N-terminus of pullulanase would not go beyond the Y region. None of the cited documents teach a truncated pullulanase that comprises a conserved Y or VWAP region.

Further more in the Advisory action, dated July 16, 2002, the Examiner stated, "However such arguments are moot as claims are not directed to identification or disclosure of conserved regions of the pullulanases." By these amendments, Applicants respectfully have identified the conserved region Y and conserved region VWAP.

Applicants believe the pending claims are in condition for allowance and issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-4020.

Respectfully submitted,



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